UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

April 1, 2003

MEMORANDUM:

Subject: Efficacy Review for EPA Reg. No.: 70144-R,"Opti-Cide 2"
DP Barcode: D287911 & D288840
Case No: 065260

From: Emily Mitchell, M.S., Team Leader
Efficacy Evaluation Team
Product Science Branch
Antimicrobials Division (7510C)

To: Marshall Swindell PM 33/Karen Leavy-Munk
Regulatory Management Branch I
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Applicant: Micro-Scientific Industries, Inc.
1225 Carnegie Street, Suite 104B
Rolling Meadows, IL 60008

Formulation From Label:

Active Ingredient(s)	% by wt.
n-Alkyl (60% C_{14}, 30% C_{16}, 5% C_{12}, 5% C_{18}) dimethyl benzyl ammonium chloride	0.154%
n-Alkyl (68% C_{12}, 32% C_{14}) dimethyl ethylbenzyl ammonium chlorides	0.154%
Isopropanol	21.000%
Inert Ingredient(s)	78.692%
Total	100.000%

I BACKGROUND

The product, Opti-Cide 2 (EPA Reg. No. 70144-R), is a new product. The applicant requested to register this "ready-to-use" product as a disinfectant for use on hard, non-porous surfaces including for use in child-care, animal-care, commercial, and hospital or medical environments. All studies were conducted at MicroBioTest, Inc. located at 105B Carpenter Drive in Sterling, Virginia 20164.

This data package contained EPA Form 8570-4 (Confidential Statement of Formula), six studies (MRID Nos. 458274-01 through 458274-06), Statements of No Data Confidentiality Claims for all 6 studies, and the proposed label.

Note: A letter from the applicant's agent (dated December 18, 2002) indicates that the product, Opti-Cide 2, is a 100% "repack" of the registered product, Burnishine Germicidal Solution (EPA Reg. No. 1130-15). This December 18, 2002 letter indicates the intention of the applicant to rely on data previously developed for this registered product.

II USE DIRECTIONS

The product is designed to be used for disinfecting hard, non-porous surfaces such as ambulance patient care surfaces, anesthesia equipment surfaces, CPR training devices, mannequins, tonometer tips, backboards, bathing units, blood pressure devices, curing lights, dental equipment surfaces/devices, gonial lenses, hard cervical collars/braces, infant care equipment (bassinets, bed railing, incubators, tables, warmers, etc.), laboratory equipment/surfaces, light lens covers, medical equipment surfaces/devices, operating room lights/tables, ophthalmic equipment surfaces/devices, oxygen hoods, patient stretchers, physical therapy equipment (such as chairs, bars, empty whirlpool tanks, etc.), animal cages (aviary, canine, feline, etc.), bed railings, cabinets, countertops, doorknobs, helmets, shower floors/walls, tables, toilets (seats, rims and other exterior surfaces), work stations, wrestling mats, optical wear (excluding contact lenses), firefighter turnout gear, diving equipment and air packs, wet/dry suits, brushes, combs, hair cutting/clipping implements, hair rollers, manicure/pedicure instruments and tables/chairs, shampoo bowls, and tanning bed surfaces. The product may be used on surfaces made of plastics (such as polycarbonate, polyvinylchloride, polypropylene and polystyrene), vinyl, stainless steel, painted surfaces, Plexiglas® and glass.

Directions on the proposed label provided the following information regarding preparation and use of the product as a disinfectant: Remove gross filth/heavy soil by cleaning surfaces with the product (i.e., apply the product to surfaces; wait 20 seconds; wipe surfaces clean using a fresh paper or cloth towel; repeat as necessary). To disinfect, apply product to visibly clean surfaces using a sponge, mop, cloth, or spray, or by dipping. Allow surfaces to remain wet for 3 minutes at room temperature (69°F/20°C). Wipe surfaces dry using a clean paper or cloth towel. For pathogenic fungicidal activity, allow surfaces to remain wet for 5 minutes prior to wiping. Discard paper towels or wash cloth towels before reusing.

Finally, the proposed label directions noted that: "This product is not to be used as a terminal sterilant/high level disinfectant on any surface or instrument that . . . ."

III AGENCY STANDARDS FOR PROPOSED CLAIMS

Disinfectants for Use on Hard Surfaces in Hospital or Medical Environments

The effectiveness of disinfectants for use on hard surfaces in hospital or medical environments must be substantiated by data derived using the AOAC Use-Dilution Method (for water soluble powders and liquid products) or the AOAC Germicidal Spray Products Test (for spray products). Sixty carriers must be tested with each of 3 product samples, representing 3 different batches, one of which is at least 60 days old, against *Salmonella choleraesuis* (ATCC 10708), *Staphylococcus aureus* (ATCC 6538), and *Pseudomonas aeruginosa* (ATCC 15442). To support products labeled as "disinfectants", killing on 59 out of 60 carriers is required to provide effectiveness at the 95% confidence level. The above Agency standards are presented in DIS/TSS-1.

Effectiveness of disinfectants against specific microorganisms other than those named in the AOAC Use-Dilution Method, AOAC Germicidal Spray Products as Disinfectants Method, AOAC Fungicidal Test, and AOAC Tuberculocidal Activity Method, but not including viruses, must be determined by either the AOAC Use-Dilution Method or the AOAC Germicidal Spray Products as Disinfectants Method. Ten carriers must be tested against each specific microorganism with each of 2 product samples, representing 2 different batches. To support products labeled as "disinfectants" for specific microorganisms (other than those microorganisms named in the above test methods), killing of the specific microorganism on all carriers is required. In addition, plate count data must be submitted for each microorganism to demonstrate that a concentration of at least 10^4 microorganisms survived the carrier-drying step. These Agency standards are also presented in DIS/TSS-1.

Disinfectants for Use as Tuberculocides (Using the AOAC Tuberculocidal Activity Test Method or the AOAC Germicidal Spray Products as Disinfectants Method)

Disinfectants may bear additional label claims of effectiveness as tuberculocides when supported by appropriate tuberculocidal effectiveness data. Products may be tested using one of four recommended methods: the AOAC Tuberculocidal Test Method, Tuberculocidal Activity of Disinfectants Test Method with significant modification of the standard test conditions of contact time and/or temperature, Quantitative Tuberculocidal Activity Test Method, and AOAC Germicidal Spray Products as Disinfectants Method.

When using the existing or modified AOAC Tuberculocidal Activity Test Methods or the AOAC Germicidal Spray Products as Disinfectants Method, 10 carriers for each of 2 samples, representing 2 different batches of product, must be tested against *Mycobacterium bovis* BCG (a member of the *Mycobacterium tuberculosis* species complex). When using the existing or modified AOAC Tuberculocidal Activity Test Method or the AOAC Germicidal Spray Products as Disinfectants Method, killing on all carriers/slides as demonstrated in Modified Proskauer-Beck Broth, and no growth in any of the inoculated tubes of 2 additional media (i.e., Middlebrook 7H9 Broth Difco B, Kirchners Medium, and/or TB Broth Base) is required. These Agency standards are presented in EPA DIS/TSS-6, Subdivision G Guidelines, and "Data Call-In Notice for Tuberculocidal Effectiveness Data for All Antimicrobial Pesticides With Tuberculocidal Claims," dated June 13, 1986.

Certain chemical classes (i.e., glutaraldehyde and quaternary ammonium compounds) are required to undergo validation testing in addition to basic testing. Products that are formulated with other chemical groups do not require validation testing. For the product being reviewed in this data package (i.e., a quaternary ammonium compound), validation data must be developed by testing 1 additional sample of the product by a laboratory of the applicant's choice (other than the laboratory which developed the original efficacy data) using the same test procedure and test conditions as the original laboratory.

Virucides

The effectiveness of virucides against specific viruses must be supported by efficacy data that simulates, to the extent possible in the laboratory, the conditions under which the product is intended to be used. Carrier methods that are modifications of either the AOAC Use-Dilution Method (for liquid disinfectants) or the AOAC Germicidal Spray Products as Disinfectants Method (for spray disinfectants) must be used in developing data for virucides intended for use upon dry inanimate, environmental surfaces (e.g., floors, tables, cleaned dried medical instruments). To simulate in-use conditions, the specific virus to be treated must be inoculated onto hard surfaces, allowed to dry, and then treated with the product according to the directions for use on the product label. One surface for each of 2 different batches of disinfectant must be tested against a recoverable virus titer of at least 10^4 from the test surface for a specified exposure period at room temperature. Then, the virus must be assayed by an appropriate virological technique, using a minimum of four determinations per each dilution assayed. Separate studies are required for each virus. The calculated viral titers must be reported with the test results. For the data to be considered acceptable, results must demonstrate complete inactivation of the virus at all dilutions. When cytotoxicity is evident, at least a 3-log reduction in titer must be demonstrated beyond the cytotoxic level. These Agency standards are presented in DIS/TSS-7.

Virucides - Novel Virus Protocol Standards

To ensure that a virus protocol has been adequately validated, data should be provided from at least 2 independent laboratories for each product tested (i.e., 2 batches per product per laboratory). The validation of a protocol requires the use of a common positive control disinfectant to be tested concurrently with all new products. For the Hepatitis C Virus protocol, the usual control is Bardac 2280, a quaternary ammonium compound obtained from Lonza, Inc. For the Hepatitis B Virus protocol, the usual control is BTC 835, a quaternary ammonium compound obtained from Stepan, Inc. These agents serve as both intra-laboratory and inter-laboratory controls and are used for analyzing the reproducibility of the efficacy data results for the respective protocols. The Agency standards for the Hepatitis C Virus protocol are tailored from those presented in the Federal Register, Vol. 65, No. 166, Friday, August 25, 2000 and from the August 6, 2002 memorandum entitled "Review of a Protocol for Testing Disinfectants against Hepatitis C Virus Using Bovine Viral Diarrhea Virus." The Agency standards for the Hepatitis B Virus protocol are tailored from those presented in the Federal Register, Vol. 65, No. 166, Friday, August 25, 2000.

Confirmatory Efficacy Data Requirements – Repackaged Products

Products proposed for registration that are merely "repacks" of a product already registered and manufactured by another applicant require only documentation of this identity and specific references to the supporting data developed for the registered product. Confirmatory data are not required for repackaged products.

IV COMMENTS ON THE SUBMITTED EFFICACY STUDIES

1. MRID 458274-01 "Germicidal Spray Test (*S. aureus*, *P. aeruginosa*, and *S. choleraesuis*) of Opti-Cide 2" by Shiva D. Rajaram. Study conducted at MicroBioTest, Inc. Study completion date – September 25, 2002.

This study was conducted against *Staphylococcus aureus* (ATCC 6538), *Salmonella choleraesuis* (ATCC 10708), and *Pseudomonas aeruginosa* (ATCC 15442) in the presence of a 5% organic soil load (heat-inactivated horse serum). Three lots (Lot Nos. 50642, 90852, and 20242) of the product, Opti-Cide 2, were tested using MicroBioTest Protocol "Germicidal Spray Test" dated March 25, 2002 (copy provided). The AOAC Official Methods of Analysis, 16th Edition, 1995 was referenced. At least one product lot (Lot No. 20242) was at least 60 days old at the time of testing. The product was received ready to use. Sixty (60) glass slide carriers per product lot were inoculated with 0.01 mL of the test culture, which was spread uniformly over a 1 x 1 inch area of each carrier. The carriers were dried for 20-40 minutes at 37±2°C. The carriers then were sprayed (at a distance of 6-8 inches) with the product until saturated. The carriers were wiped with a sterile tissue using a left to right motion. Each carrier was sprayed again (at a distance of 6-8 inches) with the product until saturated and held for 3 minutes at 21-22°C. The carriers were transferred to Letheen Broth containing 7% Polysorbate 80 and 1% Lecithin to neutralize. The subcultures were incubated for 48±2 hours at 37±2°C, and then observed for the presence or absence of visible growth. Controls included viability, confirmation of the challenge microorganisms, neutralization effectiveness, sterility, and dried carrier counts.

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

2. MRID 458274-02 "Virucidal Effectiveness Test Using Bovine Viral Diarrhea Virus (Surrogate for Human Hepatitis C Virus) of Opti-Cide 2" by Shiva D. Rajaram. Study conducted at MicroBioTest, Inc. Study completion date – June 21, 2002.

This study, under the direction of Study Director M. Khalid Ijaz, was conducted against Bovine viral diarrhea virus (BVDV-CPE; obtained from American BioResearch Laboratories, Seymour, Tennessee) using MDBK cells (obtained from American BioResearch Laboratories, Seymour, Tennessee) as the host system. The study protocol followed MicroBioTest Protocol "Virucidal Effectiveness Test Using Bovine viral diarrhea virus (Surrogate for human Hepatitis C virus)" dated March 20, 2002 (copy provided). Two lots (Lot Nos. 50642 and 90852) of the product, Opti-Cide 2, were tested. The product was received ready to use. Serum, at a concentration of at least 5%, was present in the virus stock. Two glass carriers were tested for each product lot against the target virus. Films of virus were made by spreading 0.2 mL of

stock virus on the bottoms of separate sterilized Petri dishes. The virus films were dried for 30-60 minutes at room temperature. The product was sprayed onto the carriers at a distance of 6-8 inches until thoroughly wet. Following a 30 second contact time, the carriers were wiped once with a sterile tissue. The carriers were then sprayed again with the product in the same manner and allowed to remain for 3 minutes at 20±2°C. The carriers then were neutralized with 2.0 mL of horse serum. The neutralized mixture was then scraped from the surface of the dish with a cell scraper. Each sample (0.5 mL) of neutralized mixture was loaded onto pre-spun Sephacryl S-1000 columns and spun to obtain the eluate. Ten-fold serial dilutions were prepared, using Minimum Essential Media Eagle's containing 5% horse serum. MDBK cells were inoculated with an unspecified amount of selected dilutions and incubated at 37±2°C in 5±1% CO_2 for 3-5 days. The plates were assayed by direct immunofluorescence assay. Host cells were fixed with TC grade alcohol, stained and read for infectivity, and enumerated as Most Probable Number (MPN). Controls included cell viability, virus stock titer, plate recovery, column titer, neutralizer effectiveness, cytotoxicity, and data consistency. Lonza Inc.'s Bardac 2280 (lot number not specified) was used as the data consistency control at two concentrations, 50 ppm (titrated at 50 ppm) and 350 ppm (titrated at 345.5 ppm).

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

Note: An initial test was performed on May 8, 2002. On May 13, 2002, the laboratory discovered that the cells on the plates had detached and were washed out during the fixation step. No data were available from this initial test. The laboratory repeated the test.

3. MRID 458274-03 "Confirmatory Virucidal Effectiveness Test Using Bovine Viral Diarrhea Virus (Surrogate for Human Hepatitis C Virus) of Opti-Cide 2," by Shiva D. Rajaram. Study conducted at MicroBioTest, Inc. Study completion date – June 26, 2002.

This study, under the direction of Study Director John C. Pugh, was conducted against Bovine viral diarrhea virus (BVDV-CPE; obtained from American BioResearch Laboratories, Seymour, Tennessee) using MDBK cells (obtained from American BioResearch Laboratories, Seymour, Tennessee) as the host system. The study protocol followed MicroBioTest Protocol "Confirmatory Virucidal Effectiveness Test Using Bovine viral diarrhea virus (Surrogate for human Hepatitis C virus" dated March 20, 2002 (copy provided). One lot (Lot No. 50642) of the product, Opti-Cide 2, was tested. The product was received ready to use. Serum, at a concentration of at least 5%, was present in the virus stock. Two glass carriers were tested for the product lot against the target virus. Films of virus were made by spreading 0.2 mL of stock virus on the bottoms of separate sterilized Petri dishes. The virus films were dried for 30-60 minutes at room temperature. For each product lot, the product was sprayed onto the carriers at a distance of 6-8 inches until thoroughly wet. Following a 30 second contact time, the carriers were wiped with a sterile tissue. The carriers were then sprayed again with the product in the same manner and allowed to remain for 3 minutes at 20±2°C. The carriers were neutralized with 2.0 mL of horse serum. The neutralized mixture was then scraped from the surface of the dish with a cell scraper. Each sample (0.5 mL) of the neutralized mixture was loaded onto pre-spun Sephacryl S-1000 columns and spun to obtain the eluate. Ten-fold serial dilutions were prepared, using Minimum Essential Media Eagle's containing 5% horse serum. MDBK cells were inoculated with an unspecified amount of selected dilutions and incubated at

37±2°C in 5±1% CO_2 for 3-5 days. The plates were assayed by direct immunofluorescence assay. Host cells were fixed with TC grade alcohol, stained and read for infectivity, and enumerated as Most Probable Number (MPN). Controls included cell viability, virus stock titer, plate recovery, column titer, neutralizer effectiveness, cytotoxicity, and data consistency. Lonza Inc.'s Bardac 2280 (Lot No. 5081-170A) was used as the data consistency control at two concentrations, 50 ppm (titrated at 50 ppm) and 350 ppm (titrated at 348.6 ppm).

Note: The study was conducted according to GLP with the following exception: Not all data was recorded in strict compliance with GLP standards.

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

4. MRID 458274-04 "Virucidal Effectiveness Test Using Duck Hepatitis B Virus (DHBV) of Opti-Cide 2," by Shiva D. Rajaram. Study conducted at MicroBioTest, Inc. Study completion date – July 31, 2002.

This study, under the direction of Study Director Zheng Chen, was conducted against Duck Hepatitis B Virus (DHBV; strain not specified; obtained from Hepadna Virus Testing) using primary duck hepatocytes (obtained from purchased ducklings) as the host system. The study protocol followed MicroBioTest Protocol "Virucidal Effectiveness Test Using Duck Hepatitis B Virus" dated March 20, 2002 (copy provided). Two lots (Lot Nos. 50642 and 90852) of the product, Opti-Cide 2, were tested. The product was received ready to use. Serum, at a concentration of at least 5%, was present in the virus stock. Two glass carriers were tested for each product lot against the target virus. Films of virus were made by spreading 0.2 mL of stock virus on the bottoms of separate sterilized Petri dishes. The virus films were dried for 30-60 minutes at room temperature. For each product lot, the product was sprayed onto the carriers at a distance of 6-8 inches until thoroughly wet. Following a 30 second contact time, the carriers were wiped with a sterile tissue. The carriers were then sprayed again in the same manner and allowed to remain for an additional 3 minutes at 20±2°C. The carriers were neutralized with 2.0 mL of fetal bovine serum. The neutralized mixture was then scraped from the surface of the dish with a cell scraper. Each sample (0.5 mL) of the neutralized mixture was loaded onto pre-spun Sephacryl S-1000 columns and spun to obtain the eluate. Ten-fold serial dilutions were prepared, using Liebovitz-15 complete tissue culture medium. Primary duck hepatocytes were inoculated in quadruplicate with an unspecified amount of each dilution and incubated at 37±2°C in 5±1% CO_2 for 20-30 hours for viral adsorption. The cells were then washed once with the test medium, "re-fed" with 2 mL of the test medium, and returned to incubation conditions for 7-14 days. The plates were assayed by indirect immunofluorescence assay. Host cells were fixed in TC grade alcohol, stained and read for infectivity, and enumerated as Most Probable Number (MPN). Controls included cell viability, virus stock titer, plate recovery, column titer, neutralizer effectiveness, cytotoxicity, and data consistency. Stepan, Inc.'s BTC 835 (Lot No. 7-39358) was used as the data consistency control at two concentrations, 175 ppm (titrated at 177 ppm) and 350 ppm (titrated at 357.1 ppm).

Note: The study was conducted according to GLP with the following exception: Not all data was recorded promptly in strict compliance with GLP standards.

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

Note: Reference is made to Lonza, Inc. on one of the Project Sheets [page 16 of the MRID]. The data consistency control was obtained from Stepan, Inc., not Lonza, Inc.

5. MRID 458274-05 "Confirmatory Virucidal Effectiveness Test Using Duck Hepatitis B Virus (HDBV) of Opti-Cide 2," by Shiva D. Rajaram. Study conducted at MicroBioTest, Inc. Study completion date – July 26, 2002.

This study, under the direction of Study Director John C. Pugh, was conducted against Duck Hepatitis B Virus (HDBV; strain not specified; obtained from Hepadna Virus Testing) using primary duck hepatocytes (obtained from purchased ducklings) as the host system. The study protocol followed MicroBioTest Protocol "Confirmatory Virucidal Effectiveness Test Using Duck Hepatitis B Virus" dated March 20, 2002 (copy provided). One lot (Lot No. 50642) of the product, Opti-Cide 2, was tested. The product was received ready to use. Serum, at a concentration of at least 5%, was present in the virus stock. Two glass carriers were tested for each product lot against the target virus. Films of virus were made by spreading 0.2 mL of stock virus on the bottoms of separate sterilized Petri dishes. The virus films were dried for 30-60 minutes at room temperature. The product was sprayed onto the carriers at a distance of 6-8 inches until thoroughly wet. Following a 30 second contact time, the carriers were wiped with a sterile tissue. The carriers were then sprayed again in the same manner and allowed to remain for 3 minutes at 20±2°C. The carriers were neutralized with 2.0 mL fetal bovine serum. The neutralized mixture was then scraped from the surface of the dish with a cell scraper. Each sample (0.5 mL) of the neutralized mixture was loaded onto pre-spun Sephacryl S-1000 columns and spun to obtain the eluate. Ten-fold serial dilutions were prepared, using Liebovitz-15 complete tissue culture medium. Primary duck hepatocytes were inoculated in quadruplicate with an unspecified amount of each dilution and incubated at 37±2°C in 5±1% CO_2 for 20-30 hours for viral adsorption. The cells were then washed once with the test medium, "re-fed" with 2 mL of the test medium, and returned to incubation conditions for 7-14 days. The plates were assayed by indirect immunofluorescence assay. Host cells were fixed in TC grade alcohol, stained and read for infectivity, and enumerated as Most Probable Number (MPN). Controls included cell viability, virus stock titer, plate recovery, column titer, neutralizer effectiveness, cytotoxicity, and data consistency. Stepan, Inc.'s BTC 835 (Lot No. 7-39358) was used as the data consistency control at two concentrations, 175 ppm and 350 ppm (titrated at 175.4 ppm and 350.9 ppm, respectively).

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

Note: Reference is made to Lonza, Inc. on one of the Project Sheets [page 14 of the MRID]. The data consistency control was obtained from Stepan, Inc., not Lonza, Inc.

6. MRID 458274-06 "Tuberculocidal Activity (*Mycobacterium bovis* BCG) of a Germicidal Spray, Opti-Cide 2," by Shiva D. Rajaram. Study conducted at MicroBioTest, Inc. Study completion date – November 26, 2002.

This study was conducted against *Mycobacterium bovis* BCG in the presence of a 5% organic soil load (heat-inactivated horse serum). Two lots (Lot Nos. 50642 and 90852) of the product, Opti-Cide 2, were tested using the AOAC Confirmative in vitro Test for Determining Tuberculocidal Activity Method as described in the AOAC Official Methods of Analysis, 16th Edition, 1995. The product was received ready to use. Ten (10) glass carriers for each product lot were each inoculated with 0.01 mL of the culture, which was immediately spread uniformly over a 1 x 1 inch area of each carrier. The carriers were dried for 20-40 minutes at 37±2°C. The carriers were then sprayed (at a distance of 6-8 inches) with the product until saturated. The carriers then were wiped with a sterile tissue using one left to right motion. The carriers were sprayed again (at a distance of 6-8 inches) until saturated and held for 3 minutes at 20±1°C. The excess liquid was allowed to drain. The carriers were transferred to a tube containing 20 mL Modified Proskauer-Beck Medium (MPBM) with 7% Polysorbate 80 and 1% Lecithin to be neutralized. After 10 minutes, the carriers were transferred to tubes containing 20 mL of MPBM. From each tube of neutralizer, 2 mL was subcultured to a tube containing Middlebrook 7H9 Broth (20 mL) and 2 mL was subcultured to a tube containing Kirchner's Medium (20 mL); this was repeated for all carriers. Each tube was incubated for 60 days at 37±2°C and observed for the presence or absence of visible growth. Controls included viability, neutralizer effectiveness, sterility, dried carrier count, and confirmation of the challenge microorganism.

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

7. MRID 458640-01 "Fungicidal Spray Test (*Trichophyton mentagrophytes* of Opti-Cide 2" by Felicia L. Sellers. Study conducted at MicroBioTest, Inc. Study completion date – January 15, 2003.

This study was conducted against *Trichophyton mentagrophytes* (ATCC 9533) in the presence of a 5% organic soil load (heat-inactivated horse serum). Two lots (Lot Nos. 23052, and 52822) of the product, Opti-Cide 2, were tested using MicroBioTest Protocol "Fungicidal Spray Test" dated December 6, 2002 (copy provided). The AOAC Official Methods of Analysis, 16th Edition, 1995 was referenced. The product was received ready to use. Ten (10) glass slide carriers per product lot were inoculated with 0.01 mL of the test culture, which was spread uniformly over a 1 x 1 inch area of each carrier. The carriers were dried for 30-40 minutes at 37±2°C. The carriers then were sprayed (at a distance of 6-8 inches) with the product until saturated. The carriers were wiped with a sterile tissue using a left to right motion. Each carrier was sprayed again (at a distance of 6-8 inches) with the product until saturated and held for 3 minutes at 20±1°C. The carriers were transferred to Letheen Broth containing 7% Polysorbate 80 and 1% Lecithin to neutralize. The subcultures were incubated for 10 days at 25-30°C, and then observed for the presence or absence of visible growth. Controls included viability, confirmation of the challenge microorganisms, neutralization effectiveness, sterility, and dried carrier counts.

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

V RESULTS

MRID Number	Organism	No. Exhibiting Growth/Total No. Tested			Dried Carrier Count (CFU/ carrier)
		Lot No. 50642	Lot No. 90852	Lot No. 20242	
458274-01	*Staphylococcus aureus*	0/60	0/60	0/60	1.3×10^6
	Pseudomonas aeruginosa	0/60	0/60	0/60	1.6×10^4
	Salmonella choleraesuis	0/60	1/60	0/60	5.5×10^4

MRID Number	Organism	Mean of Duplicates expressed in Log_{10}MPN/mL				
		Lot No. 50642	Lot No. 90852	Plate Recovery Control	Bardac 2280	
					50 ppm	350 ppm
458274-02	Bovine viral diarrhea virus	0.00 Complete inactivation	0.00 Complete inactivation	5.67592	3.92069	0.00
458274-03	Bovine viral diarrhea virus	0.00 Complete inactivation	—	5.67592	3.92069	0.00

MRID Number	Organism	Mean of Duplicates expressed in Log_{10}MPN/mL				
		Lot No. 50642	Lot No. 90852	Plate Recovery Control	BTC 835	
					175 ppm	350 ppm
458274-04	Duck Hepatitis B virus	0.00 Complete inactivation	0.00 Complete inactivation	4.79357	3.58538	0.00
458274-05	Duck Hepatitis B virus	0.00 No Cytotoxicity	—	4.79357	3.58538	0.00

MRID Number	Organism	Subculture Medium	No. Exhibiting Growth/Total No. Tested		Dried Carrier Count (CFU/ carrier)
			Lot No. 50642	Lot No. 90852	
458274-06	*Mycobacterium bovis* BCG	Modified Proskauer-Beck Medium	0/10 60 days 0/10 90 days	0/10 60 days 0/10 90 days	4.0×10^4
		MPBM containing 7% Polysorbate 80 and 1% Lecithin	0/10 60 days 0/10 90 days	0/10 60 days 0/10 90 days	
		Middlebrook 7H9 Broth	0/10 60 days 0/10 90 days	0/10 60 days 0/10 90 days	
		Kirchner's Medium	0/10 60 days 0/10 90 days	0/10 60 days 0/10 90 days	

MRID Number	Organism	No. Exhibiting Growth/Total No. Tested		Dried Carrier Count (CFU/ carrier)
		Lot No. 23052	Lot No. 52822	
458640-01	*Trichophyton mentagrophytes*	0/10	0/10	1.2×10^4

VI CONCLUSIONS

1. The submitted efficacy data (MRID No. 458274-02) do not support the use of the product, Opti-Cide 2, as a disinfectant with bactericidal activity when tested against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Salmonella choleraesuis* in the presence of a 5% organic soil load (heat-inactivated horse serum) on hard, non-porous surfaces for a contact time of 3 minutes. Although results demonstrated killing on at least 59 of 60 carriers, the study was significantly different from the label-specified directions. In the study, the product was applied to the carrier surfaces and allowed to remain for 30 seconds before being wiped off. The product was applied again and allowed to remain on the carrier surfaces for 3 minutes. The label-specified directions do not instruct the user to wipe the treated surface after 30 seconds. The laboratory may have been attempting to simulate pre-cleaning of surfaces with heavy soil deposits. However, the 5% organic soil load included in the test procedure is a soil load suggested by DIS/TSS-2 to represent a lightly or moderately soiled surface. A pre-cleaning step (i.e., wiping of the treated surface after 30 seconds) is not appropriate.

Note: The dried carrier counts were at least 1×10^4 CFU/carrier. Neutralization effectiveness testing showed positive growth of the organism in Letheen Broth containing 7% Polysorbate 80 and 1% Lecithin. Viability controls were positive for growth. Sterility controls exhibited no growth.

2. The submitted efficacy data (MRID Nos. 458274-02 and -03) do not support the use of the product, Opti-Cide 2, as a disinfectant with virucidal activity when tested against Bovine viral diarrhea virus (a surrogate for Hepatitis C Virus) in the presence of a 5% organic soil load (serum) on hard, non-porous surfaces for a contact time of 3 minutes. Although results demonstrated complete inactivation of the virus, the procedure used to treat the carriers in the study was significantly different from the label-specified directions. [See Item #1 for more detail.] A pre-cleaning step (i.e., wiping of the treated surface after 30 seconds) is not appropriate.

Note: No cytotoxicity was observed. Complete inactivation (no growth) was indicated in the 10^{-2} through 10^{-7} dilutions. Based on the plate recovery control, it appears that a recoverable virus titer of at least 10^{-4} was achieved. Neutralization effectiveness testing showed positive growth. The studies were performed at the same laboratory but under the direction of different study directors.

3. The submitted efficacy data (MRID Nos. 458274-04 and -05) do not support the use of the product, Opti-Cide 2, as a disinfectant with virucidal activity when tested against Duck Hepatitis B Virus in the presence of a 5% organic soil load (serum) on hard, non-porous surfaces for a contact time of 3 minutes. Although results demonstrated complete inactivation of the virus, the procedure used to treat the carriers in the study was significantly different from the label-specified directions. [See Item #1 for more detail.] A pre-cleaning step (i.e., wiping of the treated surface after 30 seconds) is not appropriate.

Note: No cytotoxicity was observed. Complete inactivation (no growth) was indicated in the 10^{-2} through 10^{-7} dilutions. Based on the plate recovery control, it appears that a recoverable virus titer of at least 10^{-4} was achieved. Neutralization effectiveness testing showed positive growth. The studies were performed at the same laboratory but under the direction of different study directors.

4. The submitted efficacy data (MRID No. 458274-06) do not support the use of the product, Opti-Cide 2 as a disinfectant with tuberculocidal activity when tested against *Mycobacterium bovis* (BCG) in the presence of a 5% organic soil load (heat-inactivated horse serum) on hard, non-porous surfaces for a contact time of 3 minutes. Although results showed no growth in the 60-day and 90-day subcultures of the carriers tested against two product lots, the applicant failed to provide validation data. For certain chemical classes (i.e., quaternary ammonium compounds), validation testing is required in addition to basic testing.

EPA's June 13, 1986 Memo, "Data Call-In Notice for Tuberculocidal Effectiveness Data for All Antimicrobial Pesticides With Tuberculocidal Claims," states: "Validation data must be developed on one additional sample of the product by a laboratory of the registrant's choice (other than the laboratory which developed the original data) using the same optional test procedure and test conditions as the original laboratory.

5. The submitted efficacy data (MRID No. 458640-01) do not support the use of the product, Opti-Cide 2, as a disinfectant with fungicidal activity when tested against *Trichophyton mentagrophytes* in the presence of a 5% organic soil load (heat-inactivated horse serum) on hard, non-porous surfaces for a contact time of 3 minutes. Although results demonstrated killing on all carriers, the study was significantly different from the label-specified directions. In the study, the product was applied to the carrier surfaces and allowed to remain for 30 seconds before being wiped off. The product was applied again and allowed to remain on the carrier surfaces for 3 minutes. The label-specified directions do not instruct the user to wipe the treated surface after 30 seconds. The laboratory may have been attempting to simulate pre-cleaning of surfaces with heavy soil deposits. However, the 5% organic soil load included in the test procedure is a soil load suggested by DIS/TSS-2 to represent a lightly or moderately soiled surface. A pre-cleaning step (i.e., wiping of the treated surface after 30 seconds) is not appropriate.

VII RECOMMENDATIONS

1. A letter from the applicant's agent (dated December 18, 2002) indicates that the product, Opti-Cide 2, is a 100% "repack" of the registered product, Burnishine Germicidal Solution (EPA Reg. No. 1130-15). This December 18, 2002 letter indicates the intention of the applicant to rely on data previously developed for this registered product. The EPA-approved label for the

product, Burnishine Germicidal Solution, claims that the product is effective against the following microorganisms at the contact times noted:

Herpes simplex II virus (G Strain)	30 seconds
HIV-1	30 seconds
Influenza virus (Strain A2/Hong Kong)	30 seconds
Polio 1 virus	3 minutes
Rhinovirus	3 minutes
Aspergillis niger	5 minutes
Candida albicans	5 minutes
Enterococcus faecalis	5 minutes
Trichophyton mentagrophytes	5 minutes

The proposed label for the product, Opti-Cide 2, claims effectiveness against each of the above-listed viruses at a contact time of 3 minutes and against each of the above-listed fungi at a contact time of 5 minutes. It appears that the proposed label claims regarding the use of the product, Opti-Cide 2, as a disinfectant against the microorganisms listed would be acceptable when the product is used on hard, non-porous surfaces at the label-specified contact times. Prior to approving the proposed label, the PM reviewer needs to verify that the product, Opti-Cide 2, is a "repack" of the product, Burnishine Germicidal Solution (EPA Reg. No. 1130-15). See Nancy Whyte's review (D287999 dated 4/03/03).

2. The proposed label claim regarding the use of the product, Opti-Cide 2, as a disinfectant against Methicillin-resistant *Staphylococcus aureus* is not acceptable. The data package provided did not include any efficacy data to support this claim. This bacterium is not listed on the product label for Burnishine Germicidal Solution (EPA Reg. No. 1130-15). Prior to approving the proposed label, regulatory should have the applicant remove all references to Methicillin-resistant *Staphylococcus aureus*. See Nancy Whyte's review (D287999 dated 4/03/03).

3. The proposed label claims (as supported by MRID Nos. 458274-01 through -06) are not acceptable regarding the use of the product, Opti-Cide 2, as a disinfectant against the following microorganisms on hard, non-porous surfaces for a contact time of 3 minutes:

Mycobacterium bovis BCG
Pseudomonas aeruginosa
Salmonella choleraesuis
Staphylococcus aureus
Bovine viral diarrhea virus (a surrogate for Human Hepatitis C virus)
Duck hepatitis B virus (a surrogate for Human Hepatitis B virus)

Although results demonstrated effectiveness of the product, the procedure used to treat the carriers in the study was significantly different from the label-specified directions. [See the Conclusions Section of this report for more detail.] In addition, the applicant did not provide validation data for the study against *Mycobacterium bovis* BCG (i.e., an additional sample was not tested by a separate laboratory as is required by EPA's June 13, 1986 Memo, "Data Call-In Notice for Tuberculocidal Effectiveness Data for All Antimicrobial Pesticides With Tuberculocidal Claims").

Note: The applicant provided efficacy data for the product, Opti-Cide 2, for the above-list microorganisms because the contact times on the EPA-approved label for the product, Burnishine Germicidal Solution (EPA Reg. No. 1130-15) were longer than desired. However, the PM reviewer should read Nancy Whyte's review for Burnishine Germicidal Solution (D287999 dated 4/03/03)